RECOGNITION OF *PHRAGMITES AUSTRALIS* SUBSP. *AMERICANUS* (POACEAE: ARUNDINOIDEAE) IN NORTH AMERICA: EVIDENCE FROM MORPHOLOGICAL AND GENETIC ANALYSES

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ABSTRACT

A new native subspecies of ***Phragmites australis*** subsp. ***americanus*** Saltonstall, P.M. Peterson & Soreng is described. The new subspecies can be separated from the introduced and Gulf Coast North American lineages of *P. australis* by having caducous leaf sheaths, ligules 1.0–1.7 mm long, lower glumes 3.0–6.5 mm long, upper glumes 5.5–11.0 mm long, lemmas 8.0–13.5 mm long, and by possessing chloroplast DNA haplotypes of A-H, S, Z, AA. The new subspecies is clearly distinguished in a PCA analysis and when bivariate plots of the morphological features are compared. Additional work is needed to morphologically distinguish the introduced from Gulf Coast lineages. *Phragmites berlandieri* is lectotypified. A key and distribution maps to the three lineages are included.

RESUMEN

Se describe una nueva subespecie nativa **Phragmites australis** subsp. **americanus** Saltonstall, P.M. Peterson & Soreng. La nueva subespecie puede separarse de los linajes introducidos en la Gulf Coast de Norte América de *P. australis* por tener vainas caducas, ligulas de 1.0–1.7 mm, glumas inferiores de 3.0–6.5 mm, glumas superiores de 5.5–11.0 mm long, lemas de 8.0–13.5 mm, y por tener haplotipos del DNA plastidial A-H, S, Z, AA. La nueva subespecie queda claramente diferenciada en un análisis de PCA y cuando se comparan gráficos bivariantes de las características morfológicas. Se necesita trabajo adicional para diferenciar morfológicamente los linajes introducidos de la Gulf Coast. Se lectotipifica *Phragmites berlandieri*. Se incluyen una clave y mapas de distribución de los tres linajes.

Phragmites Adans. is a cosmopolitan genus found throughout the world and is currently placed in the tribe Arundineae with *Arundo* L., *Hakonechloa* Makino ex Honda, and *Molinia* Schrank, the latter three genera all introduced in North America (Soreng et al. 2004; Zuloaga et al. 2003). *Phragmites* is an erect perennial grass, 2–5 m tall, that can form dense stands. A number of species, subspecies, and varieties have historically been described in the genus *Phragmites* and today four species are recognized: *P. australis* (Cav.) Trin. ex Steud., *P. karka* (Retz.) Trin. ex Steud., *P. mauritianus* Kunth, and *P. japonicus* Steud. All temperate subspecies and varieties are now included under the designation *P. australis* (Clayton 1968). Using five specimens collected in Texas and Mexico, Fournier (1877)

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distinguished a North American *Phragmites* (*P. berlandieri* E. Fourn.) from that found elsewhere in the world. Based on measurements of glumes from 28 European specimens and many North American specimens, Fernald (1932) supported this distinction of a North American variety, *P. communis var. berlandieri* (E. Fourn.) Fernald.

Recent genetic studies indicate that three genetic lineages of *Phragmites* are found in North America (Saltonstall 2002, 2003a,b). A lineage endemic to North America is found across much of Canada and in the United States, from New England and the Mid-Atlantic states across to the Pacific coast and into the southwest (Fig. 1a). Regional structuring can be found within this native lineage, with east coast, midwestern, and western populations showing different chloroplast DNA haplotypes (A-H, S, Z, AA, Saltonstall 2003a). Another lineage is found in the southern United States from Florida across to the Gulf of California, and this lineage is also found in Central America and in Asia (Fig. 1b). It is characterized by chloroplast haplotype I (hereafter referred to as the Gulf Coast lineage). A third lineage, chloroplast haplotype M, is EurAsian in origin and was likely introduced to North America since European colonization. It is found across the continent, both in areas where *Phragmites* was historically present and also in places (such as the southeastern US) where *Phragmites* is not native to the flora (Fig. 1c; Saltonstall 2002). Today, this introduced lineage is the most common type of *Phragmites* in North America and can be found in a variety of habitats including both brackish and freshwater marshes, inland fens, along the banks of rivers and lakes, and along roadsides.

With the recognition that both native and introduced populations of *Phragmites* may be present, many state and regional management authorities are now revising their *Phragmites* management strategies with a goal of preserving native populations while controlling introduced ones. This has also encouraged Natural Heritage programs to consider listing native *Phragmites* as a rare or threatened plant in a number of states. However, the appropriate level of taxonomic classification of the different lineages has not yet been clarified.

A number of qualitative characters have been suggested for distinguishing the native and introduced lineages including culm color, culm texture, and adherence of leaf sheaths to culms (Blossey 2002). While these characters appear to be correlated with ecological characteristics, they are problematic in that they are subject to observer judgment and may require observation at different times of the year. Although genetic testing can provide definitive information as to the lineage of a population, quantitative measurements of morphological features may provide a means of confirming origin in conjunction with qualitative characteristics. Robichaud and Catling (2003) performed such an analysis using *Phragmites* specimens collected in southern Ontario and found significant differences in the length of the lower glume between the native and

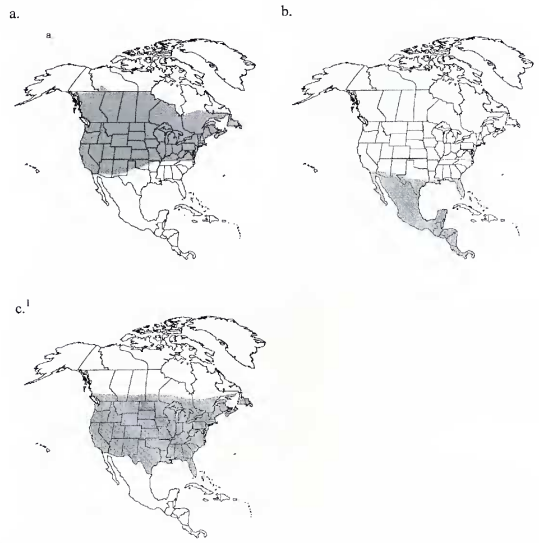

FIG. 1. Distribution of a) Native, b) Gulf Coast, and c) Introduced lineages of *Phragmites australis* in North America. Sources of distribution information include Catling et al. 2004, COMREED 1999, Saltonstall 2002.

introduced population lineages. However, that study was limited by its geographic scope. This study quantifies differences in size seen in the ligules, lower and upper glumes, and lemmas of native, introduced, and Gulf Coast populations of *Phragmites* from North America. We formally recognize the native lineage that occurs in the USA and Canada as *P. australis* subsp. *americanus* Saltonstall, P.M. Peterson & Soreng.

[1]Although not documented across the Gulf Coast except for in the Mississippi river delta (Saltonstall 2002), introduced *Phragmites* may already have invaded these regions and certainly has the potential to spread into them. The distribution of introduced *Phragmites* is not known south of the U.S. border and thus is not included in this figure.

METHODS

The genetic lineage of specimens was determined prior to taking morphological measurements. DNA extractions were done using a CTAB extraction protocol (Doyle & Dickson 1987). Lineages were identified either by sequencing two noncoding chloroplast gene regions, *trnT*(UGU)-*trnL*(UAA)5' and *rbcL-psaI*, to determine the chloroplast DNA haplotype (Saltonstall 2002) or using an RFLP diagnostic assay on the abovementioned chloroplast regions that distinguishes the three North American *Phragmites* lineages (Saltonstall 2003c).

Ligules were measured using a Nikon PZ500 dissecting microscope fitted with a 0.1–10 mm micrometer. All samples were obtained from live populations in 1999–2003 throughout the range of *Phragmites* in North America. Several leaf blades per sample were initially observed to see if there was variation in the ligule lengths. Since no within-plant or within-population variation was detected only one leaf blade was examined for the majority of specimens. Ligule length was calculated by measuring the ligule (both the membrane and its hairy margin) at the center of the leaf blade to the nearest 0.05 mm.

Measurements of lower and upper glumes and lemmas were taken from a single inflorescence per clone. Ten glumes and ten lemmas were measured from each specimen. Samples were obtained from live populations during 1999–2003 or from herbarium specimens and cover the geographic range of all *Phragmites* lineages in North America. Measurements were made to the nearest 0.5 mm using a ruler. From each specimen, ten spikelets showing visible rachilla hairs were selected from the middle part of the inflorescence. Upper and lower glume and lemma lengths were measured from the articulated base to the tip. A complete data set of the morphological characters used in this study is available from KS upon request.

Data were analyzed using the PROC MIXED procedure in SAS 8.2. Tukey's comparisons were used to distinguish significant differences between population types. Since the majority of samples measured for ligule length were different from those measured for glume and lemma lengths, the data were randomized and treated as groups representing variation within each of the three genetic lineages. Bivariate comparisons were plotted to illustrate these differences between lineages. A Principal Components Analysis (PCA) was performed using PC-ORD (Version 4, McCune & Mefford 1999) using a correlation matrix of standardized data for the variables.

RESULTS AND DISCUSSION

The morphological characters measured in this study clearly distinguish native from introduced and Gulf Coast *Phragmites* lineages. This mirrors the distinctiveness seen at the genetic level between the lineages, where all native North American haplotypes shared five unique mutations not seen in any other haplotypes (Saltonstall 2002). Native specimens have longer ligules, glumes, and lem-

TABLE 1. Mean values and their significance level for ligule, glume and lemma lengths by *Phragmites* lineages: Native (N), Introduced (I), and Gulf Coast (GC).

Structure	Population Type	Sample size	Mean ± SE (mm)	Significant Difference (p<0.01)
Ligule	Native	28	1.26 ± 0.04	I, GC
	Introduced	20	0.69 ± 0.03	N
	Gulf Coast	14	0.57 ± 0.04	N
Lower glume	Native	28	4.6 ± 0.1	I, GC
	Introduced	17	3.4 ± 0.1	N, GC
	Gulf Coast	15	3.9 ± 0.1	N, I
Upper glume	Native	28	7.3 ± 0.2	I, GC
	Introduced	17	5.8 ± 0.2	N
	Gulf Coast	15	6.3 ± 0.1	N
Lemma	Native	28	11.1 ± 0.2	I, GC
	Non-Native	17	9.2 ± 0.2	N
	Gulf Coast	15	10.1 ± 0.2	N

mas than both introduced and Gulf Coast specimens (Table 1; Ligule—$F_{2,59}$=120.21; Lower glume—$F_{2,57}$=37.59; Upper glume—$F_{2,57}$=21.01; Lemma—$F_{2,58}$=17.07; p<0.0001 for all comparisons). Of the four characters measured, the ligule is the most definitive in separating the native from the other two lineages (Fig. 2a–c). The length of the lower glume is also a good way of distinguishing native from introduced specimens, although some overlap is seen (Fig. 2d).

The Gulf Coast lineage, although significantly different from others at several measurements, is intermediate between the other two types when comparing these four characters (Table 1, Fig. 2a–d). Thus at this time, it remains difficult to distinguish morphologically and it appears premature to conclude that this lineage is a different species (Jones et al. 1997). It appears more similar to introduced than native *Phragmites* for all morphological measurements, in addition to being genetically more closely related to the introduced haplotype M than the native haplotypes (Saltonstall 2002, 2003a). Additional characters that distinguish this lineage morphologically have yet to be identified. Although not verified quantitatively, the leaf internode distance of the Gulf Coast plants appears to be shorter than both the introduced and native lineages (Saltonstall pers. obs.). The syntype of *P. berlandieri* (*J.L. Berlandier 1446*, US-82049 ex P) was included in our morphological survey and falls within the Gulf Coast lineage. Fernald (1932) did not indicate if he used one of the syntypes designated by Fournier (1877) in his study. Clearly Fernald was referring to the native lineage in his study since the lower glumes range from 4–6 mm long and the upper glumes range from 6–8.5 mm long. To avoid confusion in the future, particularly if one chooses to use the name *P. berlandieri* to include the Gulf Coast lineage, we formally lectotypify *P. berlandieri* E. Fourn., Bull. Soc. Bot. France

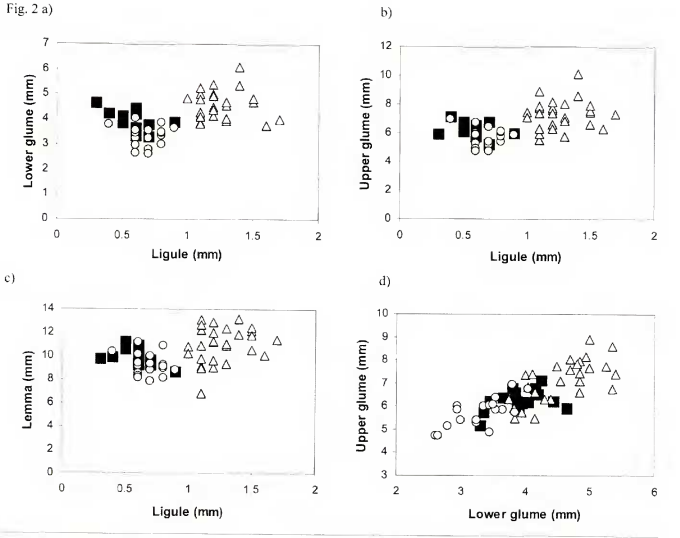

FIG. 2. Bivariate comparisons of morphological data for Native ( ), Introduced (■) and Gulf Coast (○) *Phragmites* individuals.

TABLE 2. Eigenvector loadings for the principal components (PC). Relative eigenvalues, percent of variance, and cumulative percent of variance are also listed.

	PC 1	PC 2	PC 3	PC4
Ligule	-0.4030	-0.8859	0.2293	-0.0143
Lower glume	-0.5285	0.0584	-0.7301	-0.4292
Upper glume	-0.5489	0.2219	-0.0574	0.8038
Lemma	-0.5069	0.4032	0.6411	-0.4116
Eigenvalue	2.996	0.636	0.267	0.101
% of variance	74.904	15.905	6.676	2.515
Cumulative % of variance	74.904	90.809	97.485	100.000

24:178. 1877. TYPE: U.S.A. TEXAS: Laredo, 1828, *J.L. Berlandier 1446* (LECTOTYPE: P; ISOLECTOTYPE, the large specimen on the sheet that includes a culm with a complete inflorescence: US-82049 ex W!).

The PCA confirmed and enhanced the above mentioned results further. The first two PC's accounted for 90.8% of the total variation in the data (Table 2, Fig. 3). The first axis alone accounts for 74.9% of the total variance and has negative loadings for the majority of Native specimens and positive ones for introduced and Gulf Coast specimens. Thus more negative values along PC1 indicate larger morphological structures (Fig. 2), as seen in the native specimens.

Analysis of nuclear microsatellite DNA indicates that there is little evidence for hybridization between the native and introduced lineages since alleles considered diagnostic for each of the two lineage types were rarely found in the alternative lineage. Further, this nuclear DNA dataset strongly supports the genetic differentiation seen in the chloroplast DNA between the native and introduced lineages (Saltonstall 2003b). Although *Phragmites* has been said to be self-incompatible (Gustafsson & Simak 1963), little is known about the mating system of this genus and it is not known if hybrids between population types can occur. The morphological data clearly support separation of the native lineage from the introduced/Gulf Coast lineages. We have demonstrated that the native lineage has morphological features (longer ligules, glumes, and lemmas) and unique genetic mutations that differentiate it from the introduced/Gulf Coast lineages. We chose to recognize the native lineage formally as *Pharagmites australis* subsp. *americanus*. The following key using morphological and genotype features is given to separate these three lineages.

KEY TO THE LINEAGES OF *PHRAGMITES AUSTRALIS* IN NORTH AMERICA

1. Ligules 1.0–1.7 mm long; lower glumes 3.0–6.5 mm long; upper glumes 5.5–11.0 mm long; lemmas 8.0–13.5 mm long; leaf sheaths caducous with age; culms exposed in the winter, smooth and shiny; rarely occurs in a monoculture; chloroplast DNA haplotypes A-H, S, Z, AA (see Saltonstall 2002, 2003a) ____ **P. australis** subsp. **americanus** (Native lineage)

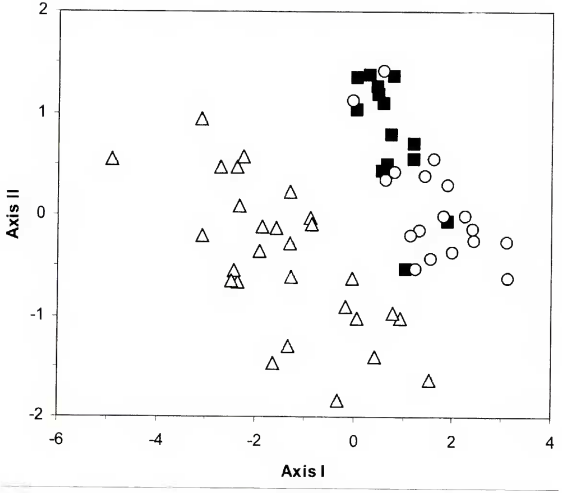

Fig. 3. Principal components analysis of morphological data for *Phragmites australis*: Native ( ), Introduced (■) and Gulf Coast (○) individuals.

1. Ligules 0.4–0.9 mm long; lower glumes 2.5–5.0 mm long; upper glumes 4.5–7.5 mm long; lemmas 7.5–12.0 mm long; leaf sheaths not caducous with age; culms not exposed in the winter, smooth and shiny or ridged and not shiny; often occurs as a monoculture; chloroplast DNA haplotypes I or M.
   2. Culms smooth and shiny; southern California, Arizona, New Mexico, Texas to Florida, throughout Mexico and Central America; chloroplast DNA haplotype I
      _____ *P. australis* var. berlandieri (E. Fourn.) C.F. Reed (Gulf Coast lineage)
   2. Culms ridged and not shiny; southern Canada from British Columbia to Quebec south throughout the Continental United States; chloroplast DNA haplotype M
      _____ *P. australis* (Introduced lineage)

Phragmites australis subsp. **americanus** Saltonstall, P.M. Peterson & Soreng, subsp. nov. TYPE: U.S.A. MONTANA. Fergus Co.: near the mouth of Dog Creek, 12 Sep 1883, *Frank Lamson Scribner 378* (HOLOTYPE: US-824621!).

A *Phragmite australi* (Cav.) Trin. ex Steud. vagina caduca cum aetate, ligulis 1.0–1.7 mm longis, glumis inferioribus 3.0–6.5 mm longis, glumis superioribus 5.5–11.0 mm longis, lemmatibus 8.0–13.5 mm longis, recedit.

Plants usually do not occur as a monoculture. Culms exposed in the winter, smooth and shiny, sometimes purplish at the nodes and internodes. Leaf sheaths caducous with age; ligules 1.0–1.7 mm mm long. Spikelet lower glumes 3.0–6.5 mm long, upper glumes 5.5–11.0 mm long; lemmas 8.0–13.5 mm long.

Distribution.—(Fig. 1a). This subspecies is known to occur in southwestern Northwest Territories east and south to California, Arizona, New Mexico, and east to northern Texas, Oklahoma, northern Arkansas, West Virginia and North Carolina, and north to Newfoundland and Quebec.

Specimens examined (included in the genetic and morphological data sets): **CANADA. BRITISH COLUMBIA:** Osoyors Lake, *J. Grant* s.n. (US-2432752). **UNITED STATES. COLORADO:** La Salle, *P.A. Rydberg 2511* (US-908102). **IOWA. Fayette Co.:** *B. Fink 592* (US-230468). **IDAHO:** St. Anthony, *E.D. Merrill & E.N. Wilcox 429* (US-908094). **INDIANA. Fulton Co.:** W of Rochester, *C.C. Deam 30010* (US-1062053). **KANSAS. Pottawatomie Co.:** *J.B.S. Norton 922* (US-353717). **MAINE:** Lake Anagunticook, Harford, *J.C. Parlin 2022* (US-908068). **MICHIGAN. Allegan Co.:** Kalamazoo River near Douglas, *W.F. Wright 125* (US-430189). **MINNESOTA:** Lake Mellissa, *H.L. Bolley 879* (US-908078). **MONTANA:** banks of the Missouri River, *F.L. Scribner 378* (US-153245). **NORTH DAKOTA. Banson Co.:** Leeds, *J. Lunell* s.n. (US-898853); **NEBRASKA. Thomas Co.:** Sand Hills near Plummer Ford, *P.A. Rydberg 1631* (US-207984). **NEW JERSEY:** New Durham, *W.M. Van Sickle* s.n. (US-244226). **NEW MEXICO:** Bremonds Ranch near Roswell, *J.D. Tinsley 12* (US-739106). **NEVADA. Nye Co.:** Amargosa Drainage Basin, *J.C. Beatley 9723* (US-2876499). **OKLAHOMA:** E of Woodward, H.E. Runyan 1030 (1722877). **OREGON:** Klammoth Co., Klammoth Lake, *E.I. Applegate 813* (US-273602). **SOUTH DAKOTA:** Canning, *D. Griffiths 105* (US-908084). **UTAH:** Rabbit Valley, *L.F. Ward 534* (US-153247). **WASHINGTON. Okanogan Co.:** Banks of the Okanogan River, *A.D.E. Elmer 519* (US-352294). **WYOMING. Fremont Co.:** Musk-Rat Creek, *L.O. Gooding 519* (US-899997).

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